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A TECHNIQUE FOR GERMINATING UREDOSPORES OF PUCCINIA STRIIFORMIS

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Robert George Emge

Crops Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

A technique for germinating uredospores of Puccinia striiformis on a modified commerial one per cent agar is described. The agar is subjected to an intensive washing procedure. With the washed agar, and with germination plates rinsed in a solution of hydrochloric acid, high germination percentages are obtained. The apparent inhibitory element in unwashed agar has not been identified.

A TECHNIQUE FOR GERMINATING UREDOSPORES OF PUCCINIA STRIIFORMIS

In initial work on stripe rust uredospore germination, we attempted to employ the techniques that had been used in the study of stem rust. It was soon apparent that stripe rust reacted differently from stem rust in many ways. Stem rust uredospores may be readily germinated on one per cent water agar. It was recorded that the germination temperatures of stripe rust uredospores fall in the range of 10° to 14°C, so these spores were dusted on the surface of one per cent water agar, placed at a favorable temperature in a saturated atmosphere for 16 to 18 hours, and germinations were noted. With this procedure, germination values rarely exceeded 20 per cent and were usually about five per cent. However, when spores of the same lot were used to inoculate plants, excellent infection was obtained. Results such as these indicated that this germination technique was not suitable. Several modifications of this technique were employed, but none produced suitable results.

At a time when we were having difficulty with the germination of stripe rust, it was learned that workers in Europe were using a purified agar for their germination work. The first test was performed with a small sample of commercially prepared purified agar. Increases in germination percentages were obtained with this sample. Other samples, identified as the same purified agar, did not give the same results. A check showed that the second sample was of a different lot number than the first. Three different lot numbers of the same material were tested, and it was found that the results with these batches did not agree (Table I). The commercially prepared agar was based on Noble's work.*

Because of the results cited above, Noble's method was modified by using granulated instead of shredded agar and glass-redistilled water in the last washing and final make-up instead of single-distilled water for the entire procedure. In brief, this modified method consists of an agar washing procedure (30 grams per two liters water) for four 24-hour periods. The agar is suspended in cold distilled water and agitated several times throughout the work day. It is allowed to set overnight and, the following morning, the liquid is decanted and discarded. Fresh distilled water is added and the procedure is repeated three times. The fourth time, redistilled water is used. After the fourth washing, redistilled water is added to bring the volume to the original two liters and the agar is melted in an autoclave. The procedure has been to divide the melted agar into portions of about 250 milliliters and store it at 4°C. Smaller amounts may be desired, depending on the quantities used for germinations, since

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* Noble, Ralph E. "A cyanide citrate pour plate medium for direct determination of the colon-aerogenes content of water and sewage," J. Am. Water Works Assoc. 19 (2) 182-192, 1928.

it has been found that repeated meltings tend to impair the solidification properties of the preparation. This material should not be remelted more then three or four times. It is also important that the washing periods not be extended beyond that specified, since excessive washing may result in failure to solidify.

During the washing procedure, it is readily apparent that something is removed in the first washing, as the liquid is a light straw color. The succeeding washings and final made-up agar are colorless. Attempts were made to identify any material removed by the washing that would materially contribute to the inhibitory influence of the unwashed agar. Chemical analyses of the washings revealed no inorganic compound or groups of compounds that would account for this inhibitory influence. Analyses for organic compounds may well produce a clue. The company that produced the material was contacted, but made no suggestions.

Although the washed agar allowed us to obtain higher germinations, we felt that they were still too low. On the basis of some former work, all germination plates were rinsed in a three to five per cent HCl solution prior to a distilled water rinse. This technique was adopted for all germination tests of stripe rust. This simple modification in washing resulted in more than a twofold increase of germination values (Table II).

The method of using washed agar and acid-washed plates for the germination of stripe rust uredospores has been adopted as a routine technique in our laboratories.

TABLE I. GERMINATION OF STRIPE RUST UREDOSPORES ON SEVERAL AGAR SUBSTRATES

Substrate	Per Cent Germination		
	Spore Lot A	Spore Lot B	Spore Lot C
1% Agar (unpurified)	10	7	13
1% Purified Agar Lot 438675	40	36	43
1% Purified Agar Lot 439335	54	45	55
1% Purified Agar Lot 444503	22	26	28
1% Detrick Washed Agar	81	78	85

TABLE II. EFFECT OF AGAR SUBSTRATE AND PLATE TREATMENT UPON THE GERMINATION OF STRIPE RUST UREDOSPORES

Substrate and Plate Treatment	Per Cent Germination
Washed agar and acid-washed plates	61
Washed agar and regular washed plates	23
Regular agar and acid-washed plates	13
Regular agar and regular washed plates	6